

SEQUENCE LISTING

<110>       Suntory Limited

<120>       Process for producing peptides using a  
            helper peptide

<130>       F962

<150>       JP 10-032272

<151>       1998-01-30

<160>       24

<210>       1

<211>       4

<212>       PRT

<213>       Artificial Sequence

<220>

<223>       Amino acid sequence adjacent to a site cleaved by  
            enterokinase

<400>       1

Asp Asp Asp Lys

1

<210>       2

<211>       4

<212>       PRT

<213>       Artificial Sequence

<220>

<223>       Amino acid sequence adjacent to a site cleaved by  
            blood coagulation Factor Xa

<400> 2  
Ile Glu Gly Arg  
1

<210> 3  
<211> 7  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Amino acid sequence containing a site cleaved by  
renin

<400> 3  
Pro Phe His Leu Leu Val Tyr  
1 5

<210> 4  
<211> 6  
<212> PRT  
<213> Artificial Sequence

<220>  
<223>

<400> 4  
Val Asp Asp Asp Asp Lys  
1 5

<210> 5  
<211> 6  
<212> PRT  
<213> Artificial Sequence

<220>

<223> Amino acid sequence of helper peptide

<400> 5

Gly Cys His His His His

1

5

<210> 6

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Amino acid sequence containing a chemically cleaved site

<400> 6

Pro Gly Gly Arg Pro Ser Arg His Lys Arg

1

5

10

<210> 7

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Amino acid sequence of helper peptide

<400> 7

His Arg His Lys Arg Ser His His His His

1

5

10

<210> 8

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> Amino acid sequence containing a site cleaved by  
Kex2 protease

<400> 8

Ser Asp His Lys Arg

1 5

<210> 9

<211> 23

<212> PRT

<213> Artificial Sequence

<220>

<223> Amino acid sequence of containing a position  
cleaved by OmpT

<400> 9

Gln Met His Gly Tyr Asp Ala Glu Leu Arg Leu Tyr Arg Arg His His

1 5 10 15

Arg Trp Gly Arg Ser Gly Ser

20

<210> 10

<211> 20

<212> PRT

<213> Artificial Sequence

<220>

<223> Amino acid sequence containing a position cleaved  
by OmpT

<400> 10

Gln Met His Gly Tyr Asp Ala Glu Leu Arg Leu Tyr Arg Arg His His  
 1 5 10 15

Gly Ser Gly Ser  
 20

<210> 11  
 <211> 69  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Nucleotide sequence coding for an amino acid  
 sequence containing a site cleaved by OmpT

<400> 11  
 cag atg cat ggt tat gac gcg gag ctc cgg ctg tat cgc cgt cat cac 48  
 Gln Met His Gly Tyr Asp Ala Glu Leu Arg Leu Tyr Arg Arg His His  
 1 5 10 15  
 cgg tgg ggt cgt tcc gga tcc 69  
 Arg Trp Gly Arg Ser Gly Ser  
 20

<210> 12  
 <211> 23  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Amino acid sequence containing a site cleaved by  
 OmpT

<400> 12  
 Gln Met His Gly Tyr Asp Ala Glu Leu Arg Leu Tyr Arg Arg His His  
 1 5 10 15  
 Arg Trp Gly Arg Ser Gly Ser  
 20

<210> 13  
 <211> 47  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Nucleotide sequence coding for an amino acid  
 sequence containing a site cleaved by OmpT  
  
 <400> 13  
 tggttatgac gcgagactcc gcctgtatcg ccgtcatcac ggttcgg 47  
  
 <210> 14  
 <211> 55  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Nucleotide sequence coding for an amino acid  
 sequence containing a site cleaved by OmpT  
  
 <400> 14  
 gatccggaac cgtgatgacg gcgatacagg cggagctccg cgtcataacc atgca 55  
  
 <210> 15  
 <211> 24  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Primer  
  
 <400> 15  
 gactcagatc ttcttgaggc cgat 24

<210> 16  
<211> 36  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Primer

<400> 16  
aaaggtacct tccgcatgcc gcggatgtcg agaagg

36

<210> 17  
<211> 20  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Primer

<400> 17  
aggccaggaa ccgtaaaaag

20

<210> 18  
<211> 29  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Primer

<400> 18  
aaaatgcatc gcatcgtaac cgtgcatct

29

<210> 19

<211> 627  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Nucleotide sequence coding for a fusion protein  
 comprising GLP-1, helper peptide and  
 $\beta$ -galactosidase protective peptide

<400> 19  
 cccaggcttt acactttatg cttccggctc gtatgttggtg tggaattgtg agcggataac 60  
 aatttcacac aggaacacagc t atg acc atg att acg gat tca ctg gcc gtc 111  
 Met Thr Met Ile Thr Asp Ser Leu Ala Val  
 1 5 10  
 gtt tta caa cgt aaa gac tgg gat aac cct gcc gtt acc caa ctt aat 159  
 Val Leu Gln Arg Lys Asp Trp Asp Asn Pro Gly Val Thr Gln Leu Asn  
 15 20 25  
 cgc ctt gca gca cat ccc cct ttc gcc agc tgg cgt aat agc gac gac 207  
 Arg Leu Ala Ala His Pro Pro Phe Ala Ser Trp Arg Asn Ser Asp Asp  
 30 35 40  
 gcc cgc acc gat cgc cct tcc caa cag ttg cgc agc ctg aat ggc gaa 255  
 Ala Arg Thr Asp Arg Pro Ser Gln Gln Leu Arg Ser Leu Asn Gly Glu  
 45 50 55  
 tgg cgc ttt gcc tgg ttt ccg gca cca gaa gcg gtg ccg gca agc ttg 303  
 Trp Arg Phe Ala Trp Phe Pro Ala Pro Glu Ala Val Pro Ala Ser Leu  
 60 65 70  
 ctg gag tca gat ctt cct gag gcc gat act gtc gtc gtc ccc tca aac 351  
 Leu Glu Ser Asp Leu Pro Glu Ala Asp Thr Val Val Val Pro Ser Asn  
 75 80 85 90  
 tgg cag atg cac ggt tac gat gcg atg cat ggt tat gac gcg gag ctc 399  
 Trp Gln Met His Gly Tyr Asp Ala Met His Gly Tyr Asp Ala Glu Leu  
 95 100 105  
 cgc ctg tat cgc cgt cat cac ggt tcc gga tcc cct tct oga cat ccg 447  
 Arg Leu Tyr Arg Arg His His Gly Ser Gly Ser Pro Ser Arg His Pro  
 110 115 120





Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly Gln Ala Ala Lys Glu  
 130 135 140

Phe Ile Ala Trp Leu Val Lys Gly Arg Gly  
 145 150

<210> 21

<211> 187

<212> PRT

<213> Artificial Sequence

<220>

<223> Amino acid sequence of a fusion protein comprising  
 GLP-1, helper peptide and  $\beta$ -galactosidase  
 protective peptide

<400> 21

Met Thr Met Ile Thr Asp Ser Leu Ala Val Val Leu Gln Arg Lys Asp  
 1 5 10 15

Trp Asp Asn Pro Gly Val Thr Gln Leu Asn Arg Leu Ala Ala His Pro  
 20 25 30

Pro Phe Ala Ser Trp Arg Asn Ser Asp Asp Ala Arg Thr Asp Arg Pro  
 35 40 45

Ser Gln Gln Leu Arg Ser Leu Asn Gly Glu Trp Arg Phe Ala Trp Phe  
 50 55 60

Pro Ala Pro Glu Ala Val Pro Ala Ser Leu Leu Glu Ser Asp Leu Pro  
 65 70 75 80

Glu Ala Asp Thr Val Val Val Pro Ser Asn Trp Gln Met His Gly Tyr  
 85 90 95

Asp Ala Pro Ile Tyr Thr Asn Val Thr Tyr Pro Ile Thr Val Asn Pro  
 100 105 110

Pro Phe Val Pro Thr Glu Pro His His His His His Gly Gly Arg Gln  
 115 120 125

Met His Gly Tyr Asp Ala Glu Leu Arg Leu Tyr Arg Arg His His Arg  
 130 135 140

Trp Gly Arg Ser Gly Ser Pro Ser Arg His Lys Arg His Ala Glu Gly  
 145 150 155 160

Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly Gln Ala Ala Lys  
165 170 175

Glu Phe Ile Ala Trp Leu Val Lys Gly Arg Gly  
180 185

<210> 22

<211> 184

<212> PRT

<213> Artificial Sequence

<220>

<223> Amino acid sequence of a fusion protein comprising  
GLP-1, helper peptide and  $\beta$ -galactosidase  
protective peptide

<400> 22

Met Thr Met Ile Thr Asp Ser Leu Ala Val Val Leu Gln Arg Lys Asp  
1 5 10 15  
Trp Asp Asn Pro Gly Val Thr Gln Leu Asn Arg Leu Ala Ala His Pro  
20 25 30  
Pro Phe Ala Ser Trp Arg Asn Ser Asp Asp Ala Arg Thr Asp Arg Pro  
35 40 45  
Ser Gln Gln Leu Arg Ser Leu Asn Gly Glu Trp Arg Phe Ala Trp Phe  
50 55 60  
Pro Ala Pro Glu Ala Val Pro Ala Ser Leu Leu Glu Ser Asp Leu Pro  
65 70 75 80  
Glu Ala Asp Thr Val Val Val Pro Ser Asn Trp Gln Met His Gly Tyr  
85 90 95  
Asp Ala Pro Ile Tyr Thr Asn Val Thr Tyr Pro Ile Thr Val Asn Pro  
100 105 110  
Pro Phe Val Pro Thr Glu Pro His His His His His Gly Gly Arg Gln  
115 120 125  
Met His Gly Tyr Asp Ala Glu Leu Arg Leu Tyr Arg Arg His His Gly  
130 135 140  
Ser Gly Ser Pro Ser Arg His Lys Arg His Ala Glu Gly Thr Phe Thr  
145 150 155 160

Ser Asp Val Ser Ser Tyr Leu Glu Gly Gln Ala Ala Lys Glu Phe Ile  
165 170 175

Ala Trp Leu Val Lys Gly Arg Gly  
180

<210> 23

<211> 184

<212> PRT

<213> Artificial Sequence

<220>

<223> Amino acid sequence of a fusion protein comprising  
GLP-1, helper peptide and  $\beta$ -galactosidase  
protective peptide

<400> 23

Met	Thr	Met	Ile	Thr	Asp	Ser	Leu	Ala	Val	Val	Leu	Gln	Arg	Lys	Asp	1	5	10	15
Trp	Asp	Asn	Pro	Gly	Val	Thr	Gln	Leu	Asn	Arg	Leu	Ala	Ala	His	Pro	20	25	30	
Pro	Phe	Ala	Ser	Trp	Arg	Asn	Ser	Asp	Asp	Ala	Arg	Thr	Asp	Arg	Pro	35	40	45	
Ser	Gln	Gln	Leu	Arg	Ser	Leu	Asn	Gly	Glu	Trp	Arg	Phe	Ala	Trp	Phe	50	55	60	
Pro	Ala	Pro	Glu	Ala	Val	Pro	Ala	Ser	Leu	Leu	Glu	Ser	Asp	Leu	Pro	65	70	75	80
Glu	Ala	Asp	Thr	Val	Val	Val	Pro	Ser	Asn	Trp	Gln	Met	His	Gly	Tyr	85	90	95	
Asp	Ala	Pro	Ile	Tyr	Thr	Asn	Val	Thr	Tyr	Pro	Ile	Thr	Val	Asn	Pro	100	105	110	
Pro	Phe	Val	Pro	Thr	Glu	Pro	His	His	His	His	His	Gly	Gly	Arg	Gln	115	120	125	
Met	His	Gly	Tyr	Asp	Ala	Glu	Leu	Arg	Leu	Tyr	Arg	Arg	His	His	Gly	130	135	140	
Ser	Gly	Ser	Pro	Ser	Arg	His	Pro	Arg	His	Ala	Glu	Gly	Thr	Phe	Thr	145	150	155	160

Ser Asp Val Ser Ser Tyr Leu Glu Gly Gln Ala Ala Lys Glu Phe Ile

165

170

175

Ala Trp Leu Val Lys Gly Arg Gly

180

<210> 24

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> Amino acid sequence containing a site cleaved by  
Kex2 Protease

<400> 24

Ser Cys His Lys Arg

1

5